Original Article

Development of ultraviolet spectroscopic method for the estimation of metronidazole benzoate from pharmaceutical formulation

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Abstract

Background: The present study was undertaken with an objective to develop a simple, accurate, cost-effective and reproducible ultraviolet spectrophotometric method for the estimation of metronidazole benzoate (MB) from pharmaceutical formulations. **Materials and Methods:** The analysis was performed on λ_{max} 268 nm by using 0.1 NHCl as diluents. The proposed method was validated on International Conference Harmonization guideline including the parameters viz., accuracy, linearity, precision, specificity and reproducibility. The proposed method was also used to access the content of MB in two commercial brands of Indian market. **Results:** Beer's law was obeyed in the concentration range of 1-10 μg/ml having regression equation y = 0.078 x-0.012.The accuracy value for 4 μg/ml and 5 μg/ml concentration of MB was found to be 99.37% and 98.9% respectively. The relative standard deviation of interday and intraday was lesser than 1%. The developed method was applied on two different marketed brands and contents of MB were found to be 98.62% and 98.59% incompliance with labeled claim. The results were under the limit of acceptance statistically. **Conclusion:** It was concluded that the proposed method canbe used for routine analysis of MB in bulk and commercial formulations.

Keywords: International Conference Harmonization guidelines, metronidazole benzoate, quality control, ultraviolet spectrometry

INTRODUCTION

Chemicallymetronidazole benzoate (MB) is 2-(2-methyl-5-nitro-1H-imidazole-1-yl) ethylbenzoate [Figure 1], belonging to the category of antiprotozoal drug, used to treat various infections including amoebicdysentery, trichomoniasis, periodontitis, giardiasis and many other anaerobic infections.[1] Asaprophylactic treatment of infection, MB is used before surgery aswellas of tenusedinoral liquid dosage for msdueto its known bitter taste.[2]

Access this article online	
Quick Response Code:	Website: www.jnsbm.org
	DOI: 10.4103/0976-9668.136154

Earlier reported method includes kinetic sp ectrophotometry,[3] high performance liquid chro matography (HPLC),[4] ultra-performance liquid chro matography,[5] LC-mass spectrometry (MS)-MS,[6] gas liquid chromatographic (GLC)[7] and differential pulse polarographic method[8] for quantification of MB from plasma, urine and milk samples. The use of HPLC, GLC and LC-MS-MS involves the use of sophisticated instruments, which requires expensive instrumentation and more time consuming steps in sample preparations. The long run time of the se methods limits their applicability for the large number of samples. The existing spectroscopic method involves tedious derivatization step, which not only increase the complexity of method, but also increases the cost and time of analysis.[9]

Upon the literature survey, it was observed that no simple, cost-effective and reproducible methods area available for the estimation of MB from bulk dosage form as well as pharmaceutical dosage form. Keeping in view this fact, focus

was laid to develop a simple, cost-effective and reproducible ultraviolet (UV) spectroscopic method for the estimation of MB and validated as per International Conference Harmonization (ICH) guideline.[10] There covery of MB by the proposed method was high, which accounts for the accuracy, novelty and reliability of the proposed method. The method was applied to determine the content of MB in two different marketed products in India.

MATERIALS AND METHODS

Instruments and materials

Shimadzu 1800 double-beam UV/V is spectrophotometer with1 cm matched quartz cells, digital balance (Citizen Co., Mumbai, India) and micropipette (The Modern Scientific Industries, Meerut, India) were used in the present study. MB was obtained as a gift sample from TTK Pharma, Vapi-Gujrat. The other chemicals and reagents were used of analytical grade.

Standard stock solution

Standard stock solution of MB was prepared by dissolving 25 mg of MB in 10 ml of 0.1 NHCl in a 25 ml volumetric flask, shaken well and finally volume was adjusted to get final concentration of 1 mg/ml. This prepared solution was used as standard stock solution.

Calibration curve

A total volume of 5 ml of 1 mg/ml aliquot solutions was further diluted up to 50 ml by 0.1 NHCl in a 100 ml volumetric flask and finally volume was adjusted up to the mark by 0.1 NHCl. Spectrophotometric scan was performed for the prepared diluted sample to find out λ_{max} in the wavelength region 200-800 nm. The λ_{max} was found to be 268 nm against blank solution [Figure 2]. From 1 mg/ml stock solution, the serial dilution pattern was followed to obtain aliquots of 1-10 μg/ml. The calibration curve was plotted between the absorbance and concentration [Figure 3]. The optical characteristics are presented in Table 1.

Sample solution preparations

The proposed method was applied to analyze three commercially available brands of MB in suspension formulations. Six bottles of MB suspensions from two different manufactures were purchased from local market of Moradabad, India. Before measuring the suspension, content of all the six bottles from two different manufacturers were mixed separately and labeled as MB1 and MB2 for the formulation of two different manufacturers. The suspension of MB equivalent to 100 mg of the drug was measured and transferred to 100 ml volumetric flask and dissolved in 20 ml 0.1 N HCl. Solution was shaken for 20 min. The resulting solution

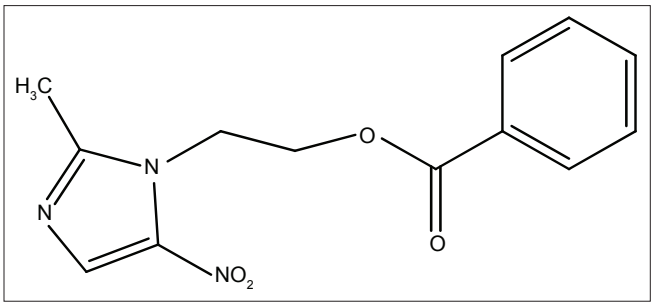

Figure 1: Chemical structure of metronidazole benzoate

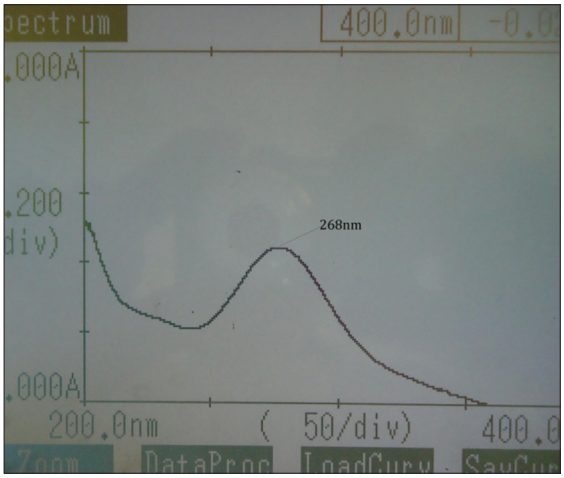

Figure 2: Scanned spectra of metronidazole benzoate

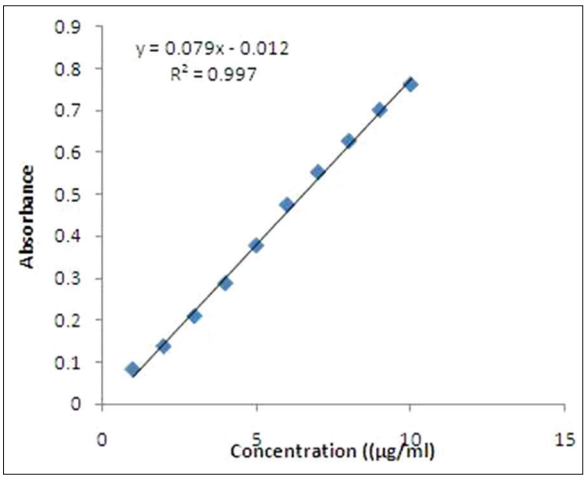

Figure 3: Calibration curve of metronidazole benzoate in 0.1N HCl

was further diluted to 100 ml with 0.1 N HCl and filtered through what man filter paper no. 41.1 ml of the above solution was pipetted out into 100 ml volumetric flask and made up to mark with 0.1 N HCl. Absorbance was measured at 268 nm against the blank solution. Finally, employing the linear equation of the calibration curve, the concentration was calculated. Percentage addition method was employed to calculate the accuracy. Amount of active drug in the different marketed brands were calculated and presented in Table 2.

RESULTS AND DISCUSSION

Linearity

The linearity of the drug was obtained for 1-10 μg/ml concentration range of MB. The calibration curve was plotted between absorbance versus concentration and the regression analysis was performed for line equation. The linear equation was found to be $y = 0.078\ x - 0.012$ and correlation coefficient (r^2) 0.997 [Table 3]. The calibration curve was found to be linear in the above stated concentration.

Accuracy (recovery)

Standard addition method was employed to access the recovery of MB in the proposed system. To get the accuracy, known amount of standard MB was added to pre-analyzed solution of MB.[11] This was carried out for 4 μg/ml and 5 μg/ml concentration in triplicate and were found to be 99.37% and 98.9% respectively [Table 2].

Precision

The precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions.[12] The precision of the assay was determined by repeatability (intraday) and intermediate precision (interday) and reported as relative standard deviation (RSD)% for a statistically significant number of replicate measurements. To determine this, 4 μg/ml and 5 μg/ml concentration solutions were measured in triplicate in a day and the same was measured in the next 3 days. The % RSD values were measured and presented in Table 2. In all cases, the RSD was always less than 2 indicating the precision of the method.

Robustness

In order to determine robustness, analysis under different temperature condition was performed, i.e., at room temperature and 22°C temperature. The absorbance of 5 μg/ml concentration was measured in triplicate at three different temperature conditions and the result is presented in % RSD [Table 4].

Ruggedness

Ruggedness of the proposed method was assessed by carrying out the analysis by different analyst as well as by changing the instrument. The result is presented in % RSD [Table 5].

Limit of detection and limit of quantification

LOD and LOQ of MB were determined by using standard deviation of the response and slope [Table 6].

Stability

The stability of MB in 0.1N HCl was studied by the proposed method. The sample solution of concentration 5 μg/ml was prepared and separately heated in order to maintain 50°C and 60°C for 60 min. Outcome of the study revealed the information about the stability of method [Table 3].

Determination of active ingredients in suspensions

This validated method was used to analyze two commercially available different brands of MB suspensions. Finding of the present amount requirement *y* revealed that both the marketed brands are incompliance with the % amount requirement (98-102%) with respect to labeled claim [Table 7].

Table 1: Calibration curve data of metronidazole benzoate in 0.1N HCl

Concentration (μg/ml)	Mean absorbance
1	0.083
2	0.138
3	0.210
4	0.288
5	0.378
6	0.475
7	0.553
8	0.627
9	0.701
10	0.762

HCl: Hydro chloric acid

CONCLUSION

The developed UV spectrophotometric method was found to be simple, cost-effective, rapid, accurate and reproducible, with high accuracy and precision values. The validation parameters were evaluated as per ICH guidelines. The satisfactory finding of this work indicates that method may be applied for quantitativeestimation of MB from pharmaceutical dosage forms. This method may be employed in routine quality control aspects.

Table 2: Determination of accuracy (by standard addition method) and precision

Ingredient	Suspension amount (μg/ml)	% addition	Amount added (μg/ml)	Amount recovered (μg/ml)	% recovery	Precision Interday	% RSD Intraday
MB	04	100	04	7.95	99.37	0.84	0.84
	05	100	05	9.89	98.90	0.38	0.65

RSD: Relative standard deviation

Table 3: Stability study

Drug concentration (µg/ml)	Absorbance at 50°C	Absorbance at 60°C	Remark
5	0.375	0.345	MB is stable upto 50°C and the degradation of MB starts on 60°C
5	0.371	0.352	
5	0.374	0.339	

MB: Metronidazole benzoate

Table 4: Robustness analysis

Concentration (µg/ml)	Absorbance at room temperature	Absorbance to 25°C
5	0.372	0.373
5	0.377	0.374
5	0.373	0.375
% RSD	<2.0	<2.0

RSD: Relative standard deviation

Table 5: Ruggedness analysis

Concentration (µg/ml)	Absorbance by analyst 1	Absorbance by analyst 2
5	0.374	0.372
5	0.371	0.373
5	0.374	0.373
5	0.375	0.375
5	0.373	0.372
% RSD	0.461 (<2.0)	0.421 (<2.0)

RSD: Relative standard deviation

Table 6: Validation parameters

Parameters	Result
Absorption maxima (λ_{max})	268 nm
Linear equation	y=0.078 x−0.012
Regression co-efficient	0.997
Linearity	1-10 (µg/ml)
LOD (µg/ml)	0.071
LOQ (µg/ml)	0.227

LOD: Limit of detection; LOQ=Limit of quantification

Table 7: Determination of active ingredients (%)

Sample	Labeled claim (mg/ml)	Amount found	% active ingredients of labeled claim
Brand 1	40	39.38	98.45
Brand 2	20	19.74	98.70

ACKNOWLEDGMENT

The authors are thankful to Dr. R. M. Dubey (Honorable Vice Chancellor), IFTM University for providing all the necessary facilities to complete this task with great ease.

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How to cite this article: Mishra AK, Kumar A, Mishra A, Mishra HV. Development of ultraviolet spectroscopic method for the estimation of metronidazole benzoate from pharmaceutical formulation. J Nat Sc Biol Med 2014;5:261-4.

Source of Support: IFTM University Administration. **Conflict of Interest:** None declared.